AUG -2 1954
AUG -2 1954
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VOLUME 12 NUMBER 8

NUTRITION REVIEWS

August 1954

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Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.
For
THE NUTRITION FOUNDATION, INC.
CHRYSLER BUILDING NEW YORK 17, N. Y.

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Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

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NUTRITION REVIEWS

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THE FOLIC ACID STORY—REMINISCENCES

The discovery of folic acid illustrates an old saying, "all roads lead to Rome." In the late thirties and early forties many groups of investigators were traveling along different roads towards the discovery of the nutritional requirements of chicks, monkeys and bacteria. The chick road was the most congested and was marked "Factor U," "Factors R and S," "Vitamin B_c," "Vitamins B_{10} and B_{11}" by the different investigators traveling along it, depending on their location, much as different parts of the same street in London have different names. The bacterial highway also had a multiplicity of names such as "norite eluate factor," "*L. casei* factor," "folic acid," and "Factor SLR." The monkey road, with much less travel, was content with the single name, "Factor M." Of all this mess of names, folic acid alone survives. This is unfortunate because it should have passed into oblivion with the other temporary markers when suitable chemical names became possible.* From folic acid has come a flock of inappropriate terms such as folinic acid, folacic acid, folinic acid-SF, none of which has any chemical derivation.

All of these roads never really met, but crisscrossed one another, so instead of Rome being a point it turned out to be an area. The names of the areas are as diverse as the roads leading to them, *viz.*, pteroylglutamic acid (PGA), N^5-formyltetrahydropteroylglutamic acid (the same as leucovorin† and folinic acid-SF), pteroic acid, rhizopterin. These names represent only parts of a whole which appears to be still unknown. Various cells require only parts of a whole, which makes the situation seem confusing and illustrates the "infinite variety" and complexity of nature in spite of man's futile efforts to make nature seem single and simple. The names are so confusing that for the past two years the reviewers of the subject in *Annual Reviews of Biochemistry* have felt obliged to define each term for the guidance of the reader. One reason for this apparent confusion is probably because no traveler has yet reached Rome. Woods, in a recent review on the subject, suggests "some form of folic acid, probably more complex in structure than any of those already known, is likely to be the ultimate coenzyme form of the group."

My modest part in the discovery of folic acid stemmed from a belief that growth-factor requirements of bacteria were inti-

* Folic acid was so named because it was obtained from a foliage material, spinach, but it is no more characteristic of leafy materials than it is of liver or yeast. Liver and yeast have been more widely used than leafy materials in the isolation and characterization of the factors; hence, if source were to determine the name, "hepatic acid" or "cerevisic acid" would have been equally logical and suitable—perhaps one should say equally illogical and unsuitable.

† The inadvisability of naming compounds because of origin or association with an agent is further illustrated by the term leucovorin, the synthetic compound that acts as a growth factor for the so-called bacterium *Leuconostoc citrovorum*, 8081. The biochemists thought their organism was an authentic *L. citrovorum*, but the bacteriologists E. A. Felton and C. F. Niven (*J. Bact.* **65**, *482 (1953)*) proved that the test agent was a typical strain of *Pediococcus cerevisiae*, so the name leucovorin has lost its significance—perhaps to the amusement of the bacteriologists and the embarrassment of the biochemists. In defense of the latter, it should be said that biochemists obtain their cultures from bacteriologists—in this case from the American Type Culture Collection—and if they are misnamed, the responsibility as well as the authority rests with the bacteriologists. Perhaps more caution and cooperation on both sides would be advantageous.

mately associated with the vitamin requirements of animals. Many others had this idea before me, among whom the most notable were Davis and Mueller. As a member of the Biochemistry Department, I was in close association with Hart, McCollum and Steenbock in the early days of vitamin research, but only as a teacher and not as a researcher. The first world war led me into fermentation biochemistry, and for about fifteen years I was more occupied with the products of bacteria than with their growth requirements. It was only when Elizabeth McCoy, E. B. Fred and I were unable to get certain clostridia (*e.g.*, *Clostridium butylicum*) to grow and produce acetone and butanol from corn meal mash, but did obtain good growth and fermentation of potato mash, that I turned my attention to the growth requirements of these bacteria. Edward L. Tatum was given this problem in 1932 for study and found that the potato mash supplied available nitrogen not present in the corn meal. Later, Snell and Lampen showed that biotin was the only vitamin required by *Cl. butylicum*. By this time the vitamin requirements of bacteria had become a major problem in our research. During the next five years a number of unusually able research men worked in our laboratories on the vitamin requirements of propionic, lactic, butyric, and hemolytic bacteria. Besides Tatum, this group included Claude Fromageot, Harland G. Wood, Esmond E. Snell, F. M. Strong, B. L. Hutchings, A. E. Oxford, D. W. Woolley, Nestor Bohonos and J. O. Lampen. I have always felt that this was the "golden age" of my scientific career.

In 1936 Tatum, Wood and Peterson published a paper showing that thiamine was required for *P. pentosaceum*. This was the first time a known vitamin had been shown to be required by bacteria, although several vitamins had already been found to be essential for the growth of yeast.

A series of papers by Snell, Strong and Peterson in 1937–1939 established the requirement of many lactic acid bacteria for riboflavin, nicotinic acid and pantothenic acid. A report on the first successful microbiologic assay (riboflavin with *Lactobacillus casei* as agent) was published by Snell and Strong in 1939. However, the existence of an unknown factor required by *L. casei* and tentatively named "norite eluate factor" was noted by E. E. Snell and W. H. Peterson (*J. Biol. Chem.* **128,** *xciv (1939)*). This eventually became known as the *L. casei* factor and also as folic acid. By this time Snell had obtained his Ph.D. degree and gone to Texas to join R. J. Williams. He continued work on bacterial growth factors there, but with another lactic acid bacterium *Streptococcus lactis R* (later shown to be a fecalis type and renamed *S. faecalis R*). In our laboratory B. L. Hutchings and Nestor Bohonos carried on the purification of the "eluate factor."

Simultaneously in the Biochemistry Department, Elvehjem, Briggs and Hegsted were working on the purification and separation of factors required by the chick. A comparison of one of the chick factors and the "norite eluate factor" showed many properties in common. Concentrates of the bacteria factor which had been purified one hundred- to two hundred-fold showed, when fed to chicks, the same concentration figure over the starting material. Likewise, treatment of the concentrate with nitrous acid resulted in inactivation for both bacteria and chicks. These results were published in a note (B. L. Hutchings and co-workers, *J. Biol. Chem.* **140,** *681 (1941)*), and provided a bridge between the two fields of nutrition. Thereafter, testing of concentrates by both chick and bacterial assay became the accepted procedure at Wisconsin and other laboratories. A detailed procedure for concentration of the factor with a description of its properties was published a few months later. By the middle of 1942 Hutchings had finished his work for the Ph.D. and gone to the Lederle Laboratories where he continued the work and had an out-

standing part in the final isolation of the pteroylglutamates (*L. casei factor*) and the establishment of their structure.

With the departure of our experienced research men and the coming of World War II, we were obliged to turn our attention to subjects of more immediate concern with national defense. Regretfully we gave up most of the work on bacterial vitamins and took up the problem of penicillin production, but this proved to be just as fascinating and fruitful as the work on vitamins.

From 1940 on, the pharmaceutical houses concentrated on the problem of isolating and characterizing folic acid. This culminated in the announcement of the isolation of two crystalline compounds, vitamin B_c by the Parke, Davis and Company workers and Factor SLR by the Merck group in 1943. Another pharmaceutical company, Lederle Laboratories, reached the ultimate goal in 1946 by isolating and establishing the structure of pteroylglutamic acid and pteroyltriglutamic acid. At the meeting of the New York Academy of Sciences announcing these achievements the late Dr. SubbaRow, the inspiring Research Director of Lederle, graciously accorded me the privilege of opening the meeting with a short paper on the "History of the Folic Acid Factors" (*Ann. N. Y. Acad. Sci.* **48,** *257 (1946)*). It was the high point of my participation in the research on folic acid. At lunch that day I said to Dr. SubbaRow, "I'd like to make a wild guess as to what the work on pteroylglutamic acid has cost Lederle. A quarter of a million dollars."

Dr. SubbaRow replied, "It has cost more than a half million." I thought wistfully, "Universities don't have that kind of money," but on second thought decided that scientifically it was magnificent that a single company was willing to put such resources and manpower into the effort.

The pharmaceutical companies have had a conspicuous part not only in the isolation and characterization of folic acid, but also more recently in the isolation and chemistry of vitamin B_{12}. Such interest and effort are understandable, since they are also the ones who reap most of the financial rewards.

In retrospect, I think my chief satisfaction comes from having been the means of guiding able young men into this field of research, in which they have accomplished so much. It is better to put ten men to work than to try to do ten men's work.

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CARBOHYDRATE IN THE TREATMENT OF DIABETIC KETOSIS

Glucose administration to patients with diabetic acidosis during insulin therapy has seemed incongruous to many physicians. It has been argued that sufficient glucose is already available and that there is no evident change in rate of recovery when glucose is given in addition to insulin. Moreover, some physicians have argued for an actual harmful effect of glucose, particularly upon the kidneys, in this situation. Another group of physicians has held that glucose administration with insulin does, in fact, speed recovery from the acidosis. Data presented by M. Rosecan and W. H. Daughaday (*J. Clin. Invest.* **33,** *49 (1954)*) show a positive therapeutic effect of both glucose and fructose upon the rate of recovery from ketonemia following insulin withdrawal. An advantage of fructose over glucose might be its presumed more rapid metabolism in the absence of insulin (*Nutrition Reviews* **9,** *173 (1951)*).

These investigators studied 8 diabetic subjects under insulin therapy. When the study began insulin was withdrawn for from one to three days, that is, until

ketonemia (averaging 5.1 millimoles per liter) was obtained. All subjects were then given insulin, the same dose for all studies in each patient being determined as that quantity which, in the course of six hours, brought the blood sugar to normal with saline but without carbohydrate administration. The experiment was repeated in the same patients once or twice with either glucose or fructose administered intravenously instead of saline. The carbohydrate was given at the rate of 0.8 g. per kilogram per hour. Standard methods were used to determine the blood and urine values for the sugars and ketones.

When sugar was given over the four-hour period there was an initial rise in blood sugar followed by a relatively constant fall, so that at the end of six hours blood sugar was considerably below the initial value but still above normal and above the value six hours after insulin and saline administration alone. The fall in blood sugar after fructose administration was more rapid than after glucose administration, but in both instances most of the sugar in the blood was present as glucose, only a small quantity being fructose. Fructose actually disappeared from the blood soon after the infusion was completed.

When saline alone was given with the insulin, negative sugar balance occurred, whereas when glucose or fructose was given sugar was retained, in a greater amount after fructose than after glucose administration.

The fall in blood ketones after insulin administration was more rapid when carbohydrate was given than when saline alone was administered. Although those patients given carbohydrate happened to have somewhat higher initial blood ketone values at the end of six hours, the final values were lower after carbohydrate was given than when none was given. The authors believe that the more rapid decline in blood ketone levels after carbohydrate administration was accounted for primarily by diminished production of ketones by the liver. There was no increase of ketone excretion in the urine and evidence largely from the work of others would not support an increased utilization of ketones in the periphery after insulin administration. Rosecan and Daughaday felt that the increased utilization of sugar (sugar retention with a falling blood sugar) induced the more rapid recovery from ketosis.

Fructose administration has been recommended by others for the treatment of diabetic ketosis. The work reported by Rosecan and Daughaday supports this advice, but as they point out "insulin is necessary for the full anti-ketogenic effect." That is, in the absence of insulin there was conversion of fructose to glucose, a subsequent rise in blood sugar and a markedly diminished tissue uptake of sugar.

The evidence presented in this study supports the concept that carbohydrate in addition to insulin produces a more rapid decrease in ketosis than does insulin alone in the uncontrolled diabetic. This is due to an increased utilization of the sugar and is somewhat better when fructose rather than glucose is given.

VITAMIN B_6 AND NEURITIS PRODUCED BY ISONIAZID

The chemotherapeutic effectiveness of isoniazid (isonicotinic acid hydrazide, or INH) in tuberculosis has given rise to speculation that the action of the drug might be related to competition with niacin. Although the mechanism of the action of this drug has not been established, this hypothesis does not appear likely. The possibility, however, that some of the toxic manifestations of INH might represent conditioned vitamin deficiency has elicited considerable interest. Peripheral neuritis occurs in about 40 per

cent of the patients receiving 20 mg. per kilogram of body weight per day of INH. This is characterized by paresthesia and numbness of the fingers and toes which proceeds centrally. Soreness and weakness of the muscles may occur. On examination the vibratory sense may be found to be impaired and decreased touch sensation may be demonstrable. The changes in the reflexes are variable.

Two severe examples of the neuritis due to isoniazid have been described by W. A. Jones and G. P. Jones (*Lancet* **I,** *1073 (1953)*). The first case was associated with mental changes and dermatitis suggesting pellagra. Large doses of thiamine had no influence on the lesions. Rapid improvement occurred following the administration of niacinamide. No therapy was attempted in the second case. In interpreting their observations the authors suggest that these complications of isoniazid therapy are due to a deficiency of niacinamide and possibly of other members of the vitamin-B_2 complex.

The possible importance of vitamin B_6 deficiency in the etiology of isoniazid neuritis is indicated by the studies of J. P. Biehl and R. W. Vilter (*Proc. Soc. Exp. Biol. Med.* **85,** *389 (1954)*). Among 36 patients receiving 20 mg. per kilogram of body weight per day of isoniazid 14 developed evidences of neuritis. Most of the cases occurred between the fourth and sixth weeks. A second group of patients given a similar dose of INH received in addition 150 to 450 mg. of pyridoxine daily beginning with the start of the antitubercular therapy. During the ten-week period of observation none of these patients developed neuritis. When pyridoxine was given at the onset of symptoms to 2 patients, no benefit resulted. Neither did the late residual neuritis respond to pyridoxine, thiamine, or niacin.

The effect of INH on the excretion of pyridoxine was studied in view of the protective action of this vitamin in preventing the occurrence of neuritis. A marked increase in the urinary excretion of pyridoxine, measured microbiologically, was found after the ingestion of INH. Following the discontinuation of the drug the excretion of pyridoxine returned to normal. Para-aminosalicylic acid, another antitubercular drug, was without effect on pyridoxine excretion. Pyridoxic acid and N_1-methylniacinamide were not excreted in increased amounts during INH therapy.

Further evidence of pyridoxine deficiency was obtained by a tryptophan-loading test. It has been found that a rise in the excretion of xanthurenic acid to 30 mg. per twenty-four hours after the oral administration of 10 g. of DL-tryptophan is suggestive of an impairment of vitamin B_6 action (H. S. Glazer and co-workers, *Arch. Biochem. Biophys.* **33,** *243 (1951)*). Loading tests were performed before and at various intervals during treatment. Marked increases in excretion were noted in many of these patients, but the response was erratic. Moreover, in several patients high levels of excretion occurred even before the administration of INH. When vitamin B_6 was given to 2 patients a significant drop in excretion of xanthurenic acid occurred. The third patient who received pyridoxine had a relatively low excretion of xanthurenic acid before treatment and it remained unchanged after the addition of this agent. In view of the variability of the results it is difficult to interpret these latter observations.

In commenting on the etiology of neuritis in INH therapy Biehl and Vilter discount the importance of metabolic antagonism to niacin. They point out that pellagra is not a recognized part of the syndrome, and moreover neuritis is not a manifestation of niacin deficiency in man. The close resemblance between the neuritis presented by patients receiving INH and that seen in those given the vitamin-B_6 antagonist, desoxypyridoxine, was what suggested a study of pyridoxine metabolism in these patients. The absence of burning sensations in the mouth and tongue, seborrheic dermatitis and

cheilosis, prominent features of desoxypyridoxine effects in man (R. W. Vilter *et al.*, *J. Lab. Clin. Med.* **42,** *335 (1953)*; *Nutrition Reviews* **12,** *10 (1954)*) weakens the argument favoring a deficiency of pyridoxine induced by INH. Two possible mechanisms by which INH could condition a deficiency of pyridoxine were suggested: (1) a disorder in renal conservation of vitamin B_6, or (2) a chemical alteration of this vitamin by INH and its excretion. It is known that pyridoxine is rapidly inactivated by hydrazines, and the possibility is mentioned that pyridoxal isonicotinyl hydrazone may have been formed and excreted in the urine. This compound would presumably be hydrolyzed in the preparation of filtrates for vitamin B_6 assay.

The observations of Biehl and Vilter appear to be of considerable importance. If confirmed, they will provide a means of controlling a troublesome complication of therapy. Of broader significance is the possible induction of vitamin deficiency by a therapeutic agent.

CHOLESTEROL SYNTHESIS IN MAN

The synthesis of cholesterol from acetate by mammals has been well demonstrated (*Nutrition Reviews* **7,** *233 (1949)*; **10,** *345 (1952)*), but the rate of cholesterol synthesis and degradation in man has remained essentially unknown. Information on this subject is of great theoretic interest as well as important in elucidating the relative importance of ingested cholesterol and its synthesis in the body in the development of arteriosclerosis.

L. Hellman, R. S. Rosenfeld, and T. F. Gallagher (*J. Clin. Invest.* **33,** *142 (1954)*) studied 6 patients with "limited life expectancy," 5 having malignant tumors and one a refractory aplastic anemia. No clinical evidence of a disturbance in cholesterol metabolism was found in any of these patients whose ages ranged from 31 to 59. Two were males and 4 were females. C^{14}-labeled acetate was given by mouth (16 mg. equivalent to approximately 200 microcuries of C^{14}). One patient was studied on two occasions.

The absorption and rapid metabolism of the acetate was demonstrated by the appearance in two hours after the administration of acetate of maximum specific activity in the exhaled carbon dioxide. Free cholesterol was rapidly formed as measured by C^{14}-labeled plasma cholesterol. Its concentration in the plasma then gradually declined over the course of approximately thirty-five days of study. The ester cholesterol on the other hand was much more gradually formed and its curve intersected the falling free cholesterol curve about two days after acetate administration.

The authors calculated that about 3 per cent (or perhaps more) of the acetate administered was incorporated into cholesterol in the plasma, red blood cells and liver, these representing the main deposits of cholesterol. Because the many possible sites of metabolism and disappearance of cholesterol were difficult to study in these patients, the authors were unable to give accurate data concerning cholesterol turnover. The data did suggest that the incorporation of the single dose of acetate into cholesterol was complete after 0.3 day. Further appearance of radioactivity was in the ester fraction and was presumed to be derived from the free sterol. This fact leads to further substantiation of the derivation of plasma esterified cholesterol from the free cholesterol fraction.

In summary, cholesterol, both free and ester, in the plasma was synthesized in man from orally administered C^{14}-labeled sodium acetate, approximately 3 per cent of the administered acetate being incorporated.

THE SODIUM-RETAINING CORTICOID IN NEPHROSIS

A large body of evidence gathered in recent years identifies the adrenal cortex as the source of a sodium-retaining hormone presumed to be active in normal individuals during dietary sodium restriction or upon exposure to high environmental temperatures, and in certain diseases such as congestive heart failure, cirrhosis of the liver and nephrosis (*Nutrition Reviews* **8,** *69 (1950)*; **10,** *287 (1952)*). For some time the synthetic material desoxycorticosterone acetate was thought to be the true adrenal cortical salt-retaining hormone, but it has not been possible to establish this, and some doubt has been cast that this substance actually exists *in vivo*. The finding by J. A. Luetscher and B. B. Johnson (*J. Clin. Invest.* **33,** *276 (1954)*) of a steroid presumably of adrenal cortical origin with sodium-retaining activity fifteen to twenty times that of desoxycorticosterone and derived from the urine of patients with nephrosis goes a long way toward establishing the relationship of the adrenal cortex to sodium retention.

Five children with nephrosis and marked sodium retention were compared to 4 normal male children. The dietary intake of sodium was between 5 and 17 milliequivalents (mEq) per day in the patients but unrestricted in the controls. Urine collections of from one to five days were pooled and extracted with chloroform after treatment with strong acid and then further purified. The extracts were then analyzed by paper chromatography and compared to known steroids. The sodium-retaining activity of the extracts was determined by bioassay and compared to desoxycorticosterone acetate. Various methods of separation were attempted, the best being a benzene:aqueous methanol system. Little or no activity was found in the extracts from normal urine. From the various reactions of the steroid tentative suggestions as to its chemical nature were made. The material was thought to be similar if not identical to some of the more recently isolated sodium-retaining steroids from the adrenal cortex (H. M. Grundy, S. A. Simpson, and J. F. Tait, *Nature* **169,** *795 (1952)*). This hormone, unlike other adrenal steroids, did not appear to be under the control of the adrenal cortex.

Clearly, the authors have isolated in relatively pure form biologically a sodium-retaining activity from the urine presumably due to an adrenal corticoid with many times the activity of desoxycorticosterone acetate.

AN APPRAISAL OF CANADIAN NUTRITURE

A comprehensive picture of the nutritional state of a population is of importance to the professional medical and governmental authorities responsible for matters of public health. It provides the basis for agricultural policies and educational efforts. It should be useful in planning nutritional research designed to obtain additional facts needed for a more accurate portrait of the current nutriture and an objective appraisal of the effectiveness of proposed remedial measures. Such an appraisal of the state of nutrition of the Canadian people has been attempted by E. G. Young (*Canad. Bull. Nutrition* **3,** *No. 1 (1953)*).

The initial step was to assemble statistics on the foods moving into civilian consumption. The figures representing national averages (totals of available foods divided by the size of the population) indicate that the Canadians have available adequate quantities of all essential nutrients. This conclusion

is valid after allowance has been made for losses of edible foods during distribution, preparation and cooking. The conclusion was based on comparisons with the Canadian dietary standards (*Canad. Bull. Nutrition* **2,** *6 (1950)*) and the (U. S.) National Research Council recommended allowances.

Consideration of the estimated supplies of foods available during the years 1935 to 1950 indicates that the Canadian dietary pattern has not changed dramatically over this period. Worthy of mention is the greater consumption of milk solids, meat and eggs, with a decrease in the consumption of cereals (from 208 pounds per person in 1935–1939 to 173 pounds in 1950). Increased use of milk, tomatoes and citrus fruits resulted in higher intakes of calcium, riboflavin and ascorbic acid. The decrements in the consumption of sugar during the war and of fats during the early postwar years were temporary. The estimated supplies of nutrients, per individual per day, moving at the level of retail stores in 1945–1950 were as follows: 3058 calories, 98 g. protein, 120 g. fat, 404 g. carbohydrate, 1.05 g. calcium, 13.5 mg. iron, 6750 I.U. vitamin A, 1.70 mg. thiamine, 2.12 mg. riboflavin, 17.3 mg. niacin and 94 mg. ascorbic acid. These are over-all figures. Observations on the distribution for the various parts of the country are fragmentary.

Dietary surveys are only gross indicators of the national consumption of essential nutrients and of nutritional status. They must be supplemented by nutrition surveys. The author reviews the available evidence, with emphasis on the work done between 1940 and 1950. A broad picture of recent nutriture in Canada has been given by L. B. Pett (*Canad. Med. Assn. J.* **63,** *1 (1950)*) who summarized data for the total of over 6000 individuals. The diets were classified as inadequate in 18 per cent of the cases on the basis of current dietary standards. It should be noted, however, that many individuals in this dietary category appeared to be perfectly healthy and showed no evidence of malnutrition. This fact should be considered whenever dietary intake and dietary standards alone are used as an "index" of nutritional status. In most instances it is, at best, only suggestive evidence.

As far as the incidence of deficiency symptoms in the Canadian on inadequate diets is concerned, underweight was recorded in 13.8 per cent and anemia in 6.8 per cent (Young, *loc. cit.*). Presence of protein deficiency was negligible, while past rickets was diagnosed in 14.5 per cent of these individuals. Symptoms of vitamin deficiencies were noted in 26.7 per cent for riboflavin, 12.8 per cent for vitamin A, and 3.6 per cent of the inadequate-diet group for ascorbic acid. The incidence of riboflavin deficiency appears high, but it was noted that the criteria, such as the "cobblestone" tongue, may be caused by factors other than riboflavin and that the incidence value was probably too high.

Data on infant and maternal mortality are given, but their relevance is questionable in consideration of the effects of the Canadian diet. Young comments that several factors, other than food, control the infant mortality levels. The table on the incidence of deaths due to avitaminosis is of interest in particular because of the continued decline in the incidence of rickets. Following the advent of irradiated ergosterol, the annual death rate from rickets was decreased from 200 in 1930 to 102 in 1931; in 1948 the value decreased to 11.

The level of blood hemoglobin has been used frequently as an index of nutriture on the assumption that low levels of hemoglobin are indicative of malnutrition. Recognizing the difference in opinion on the level of hemoglobin which constitutes anemia, Young estimates the condition of mild anemia to occur in less than 5 per cent of school children between the ages of 5 and 14 and in young women. In certain sections of the country the incidence may be much higher.

Up to the present, Canada has been

deficient in published anthropometric data on both children and adults. A study, designed to provide such data, is now being carried on. Comparison of large samples of Toronto children measured in 1923 and 1939 indicates substantial increments in body size. For 10 year old boys the mean heights in the two periods were 52.1 and 53.6 inches, for girls 49.9 and 51.4 inches; the mean weights increased from 64.6 to 68.8 pounds for boys, and for girls from 63.3 to 67.8 pounds. The differences were equivalent, in different age groups, to increments representing six months to a year of natural growth.

Of interest are several fairly recent surveys on height and weight. Again, the quantitative definition of overweight ("obesity") varies widely. E. W. McHenry, R. Crawford, and L. Barber (*Canad. J. Pub. Health* **38,** *437 (1947)*) defined overweight and underweight as deviations of more than ±10 per cent of the average weight for height, age and sex, using the 1912 Medico-Actuarial Mortality Study as reference. On this basis, out of 8075 men 15 per cent were "underweight," and 18 per cent were "overweight." For 4318 women the percentages were 26 and 15, respectively. Other authors apply the same deviation of ±10 per cent as the criterion of "abnormal" weight, but use the standard (or "ideal") weights for young adults, 25 to 30 years old, as the reference value. In some studies on mortality and overweight the average weights for age 37 at various heights according to the 1912 tables were used as the standard of reference. It is through morbidity and mortality investigations that the biologic significance of the deviation from "normal" weight must be established. Confusion in the reference standards complicates this important task.

Young (*loc. cit.*) concludes that about one fifth of the population over 30 years of age suffers from overweight which is considered as the most common form of malnutrition. The size of the population included in the overweight category depends on the limits of "normality" which, as has been pointed out, are at present a matter of opinion. The prevalence and severity of overweight is a very important point on which far-reaching medico-educational policies are based. Additional data are needed to evaluate more adequately the importance of deviations in weight within the more normal range (±20 per cent of the "standard"). Also, the ever faster growth of children, credited to the science and art of nutrition, may turn out to be a questionable blessing. This is one of the most crucial areas for research in human nutrition.

DENTAL DECAY AND CARBOHYDRATES

During the last several centuries there has been a rapid increase in the incidence of tooth decay among the populations of the western nations. Even in the last twenty years there are indications that the incidence of tooth decay has continued to mount in the United State despite the development and application of a wide variety of masterful restorative technics (P. J. Brekhus, *J. Am. Dent. Assn.* **42,** *424 (1951)*). According to records of business income, a total of $1,600,000,000 was spent during the year ending July 1, 1953 in the United States for dental care (*Ibid.* **48,** *347 (1954)*). This figure represents 15.6 per cent of the total annual expenditure for medical care of all types, including health insurance, hospitalization, nursing, etc. Unquestionably, this represents a greater financial outlay by the American nation for dental care than for any other medical specialty.

There has been a widespread belief among members of the dental profession around the world that the increasing incidence of dental caries is closely correlated with the advance of civilization and with the food pattern of

civilized races. As part of this line of reasoning, carbohydrates, and particularly sugars, have been widely incriminated as the principal causative agents of tooth decay. Actually even our knowledge of the etiology of dental caries in experimental animals is sufficiently inadequate as to make any specific statement about this effect rather tenuous at the present time unless the statement is encumbered with a substantial series of qualifications as to the circumstances involved.

Though there have been many studies on the effects of various levels of refined carbohydrates in the diet of human beings, experimental procedures and data were much less exacting than one would desire (Nutrition and Dental Caries, *p. 415*, and Oral Environment and Dental Caries, *p. 243*, *in* "A Survey of the Literature of Dental Caries," *National Research Council, Washington (1952)*). Undoubtedly the largest and the most intensive study that has been conducted on this subject was the one made in a mental institution at Vipeholm, Sweden, from 1946 until the present time, the results of which have been recently published for the years 1946–1951 (B. E. Gustafsson and collaborators, *Svensk Tandläkare-Tidskrift* **45,** *suppl. (1952)*). The main purpose of the Vipeholm dental investigation was to study certain general principles with regard to the determination of whether there was a correlation between sugar intake and dental decay and not to test the specific role of any single sugar-containing food substance. Furthermore, the investigators were interested in knowing whether or not the form in which the sugar was fed would be a determining factor in the alteration of the caries incidence and to what extent the individual caries susceptibility of the subjects would determine any carbohydrate influence. They attempted to answer the above questions as to whether caries activity was influenced under controlled conditions such as: (1) through the addition of refined sugar to the meals in forms with little tendency to be retained in the mouth; (2) through the addition with the meals of sugar in a form which would have a strong tendency to be retained in the mouth; and (3) through the addition between meals of sugar which would have a strong tendency to be retained in the mouth in certain sticky forms of candy.

Altogether there were seven groups of subjects. Groups 1, 2, 4, 5 and 6 were composed of men only while groups 3 and 7 were approximately half of each sex. At the beginning of the experiment in the fall of 1946 the total incidence of tooth decay during the lifetime of each individual was determined. Then all seven groups were maintained on the usual institutional diet for about nine months to determine the incidence of new carious lesions at that particular period of their lives. This diet was stated to be composed of typical Swedish foods in a normal ratio with no attempt to reduce drastically the carbohydrate content beyond what would be consumed by normal individuals. Eating between meals was strictly prohibited.

After this standardization period, the control group of 60 individuals with an average age of 34.9 years was maintained on the basal diet plus 150 g. of margarine per day for the first two years of the experiment; then the supplement was reduced to 40 g. of margarine per day for the second two years. These supplements were provided in order to supply a roughly comparable amount of calories to compensate for those provided to the experimental groups as sugar or candy. The second group of 57 individuals whose average age was 34.7 years was given the basal diet plus 300 g. of sucrose in solution per day for two years and then 75 g. per day for the next two years. During the full four years the sugar solution was given with the meals only. The third group of 41 male and 42 female subjects whose average ages were 30.4 and 28.0 years, respectively, was fed 345 g. of sugar-containing bread per day with the meals. The 47 subjects of an average age of 29.1 years in the fourth group received 300 g. of sucrose in solution per day

with the meals for the first two years and 65 g. of milk chocolate between meals for the second period of two years. In the fifth group, the 62 individuals (average age, 35.6 years) were given 345 g. of bread containing sugar for the first year. During the second year the bread was discontinued and the subjects were given 22 pieces of sticky caramels between meals and 200 g. of sucrose in solution. In the third year only the 22 pieces of caramel were given per day. In the fourth year the subjects were maintained on the basal diet plus 40 g. of margarine. The 40 subjects in the sixth group, who were on the average 26.3 years old, were given the basal diet plus 150 g. of margarine per day for the first year. For the next year they were given 8 toffees between meals and 250 g. of sucrose in solution per day. During the third and fourth years, the sucrose in solution was reduced to 25 g., but the toffees were continued. The 48 males and 39 females in group 7, whose ages were 31.0 and 31.1 years, respectively, were fed 24 toffees between meals and 150 g. of sucrose in solution per day for the first two years and the basal diet plus 40 g. of margarine for the second two years.

These rates of sugar consumption were unusually high in terms of what are considered to be average levels of consumption. For example, each individual who received 345 g. of sucrose in solution per day with meals consumed approximately 110 kg. of sucrose per year. In the so-called caramel group, each individual consumed approximately 73 kg.; in the group receiving 8 toffees and the sugar supplement the amount was about 92 kg.; in the experiment with the 24 toffees plus 150 g. of sucrose per day, the sugar consumption was about 55 kg. per year.

The results of these experimental groups can be described best in terms of the curves in Figure 1. These figures are expressed in terms of decayed, missing and filled (DMF) teeth per subject. The broken lines represent the results obtained when a source of carbohydrate was fed with the meals only; the solid lines represent the results when a source of carbohydrate was given between meals. The individuals in the control group were indicated to have had an average increase of less than one new DMF tooth during the five years of the observation period. Obviously this is a remarkably low rate of incidence for new diseased areas. The other six groups showed comparably low incidences of new DMF teeth in the control period of nine months.

In the second group, the increment of new DMF teeth during the first two experimental years when 300 g. of sugar was fed per day was somewhat higher than the incidence in the control period and also than that of the second two experimental years during which only 75 g. of sugar was eaten per day. However, the total gain over the four experimental years was still only slightly more than one new DMF tooth per individual.

The results of the third group were expressed as separate averages for males and females. There was a markedly higher initial DMF rate for the males than for the females. Regardless of this initial difference, the

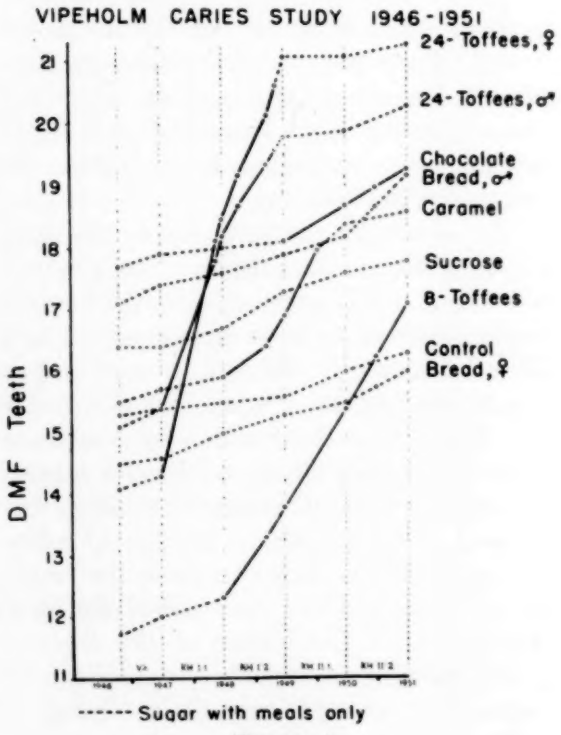

FIGURE 1

increments in new DMF teeth were approximately the same for the two subgroups fed the bread supplement over the four-year experimental period. In both subgroups the increment of new carious teeth was slightly greater than for the control group and roughly comparable to the second group which was fed the high amount of sucrose in solution. In the fourth group there was a slightly lower incidence of tooth decay during the two years in which 300 g. of sucrose solution was fed than in the control period. When the 65 g. of milk chocolate was fed there was a definitely increased tendency for new lesions to develop, as an average of 1.0 new DMF teeth was observed during these two years.

In the year when 345 g. of bread was fed per day to the subjects in the fifth group there was not any different caries increment than in the control period. However, the supplement of 22 caramels and 200 g. of sucrose in solution resulted in an average of 1.0 new DMF teeth in that year. During the next year while only 22 caramels were fed without any sugar in solution, a somewhat larger increment of lesions occurred with an average accumulation of an additional 1.4 new DMF teeth per individual in that period. For the final year with no added carbohydrate, an increment of 0.2 DMF teeth (a value comparable to the initial control period) was observed.

The results for the first year in the sixth group where no carbohydrate supplement was given were comparable to the control period. During the year that 8 toffees and 250 g. of sucrose in solution were given per day, there was an average increase of 1.5 new DMF teeth. A roughly similar increase occurred in the third and fourth experimental years when the sugar in solution was reduced to 25 g. with no change in toffee consumption. For this two years the increment of new lesions was about 3.3 new carious teeth. The results in the seventh group were again reported separately for males and females. During the two years in which 24 toffees and 150 g. of sucrose in solution were consumed per day, there was an increase in carious teeth of 4.7 for the males and 7.1 for the females. As soon as these supplements were stopped, a slower increment of new carious lesions was observed for the next two years which was comparable to the original control values.

It is noteworthy also, as shown in Figure 1, that the initial evidence of tooth decay, that is, the lifetime accumulation of tooth decay prior to the beginning of the experiment, varied tremendously within the several groups. However, the average number of new lesions which occurred in each group during the initial control period was roughly the same for each of the groups. Likewise, other periods when no carbohydrates were being fed had low increments of carious lesions which were comparable to the initial control period. The groups of individuals who consumed the toffees unquestionably had had the greatest increase in new lesions per year of any of the experimental groups. Sugar in the form of caramels or chocolate appeared to cause somewhat lesser but still major increases in the amount of tooth decay than was produced by the toffees. In contrast, the amount of tooth decay per individual per year was appreciably less in those receiving the sugar solutions alone or bread containing sugar even though tremendous amounts of carbohydrate were consumed. The latter means of providing carbohydrates in high amount seemed to have caused little or no increase in tooth decay beyond the control levels.

From these data the investigators made the logical conclusions that the addition of sugar under certain situations to an institutional diet does cause an increase in caries activity and that the chance of increasing the caries activity was greatest if the sugar was introduced in a form with a strong tendency to be retained in the mouth. Attention should also be drawn to the fact that removal of the carbohydrate from the diet resulted in a rapid return to the rate of caries development which was typical of the pre-experimental experience for the group.

This clearly indicates that the innate caries-susceptibility of the individuals within a group had not been altered.

One of the most significant demonstrations of the entire work was the vast difference in response of the individuals within any group to the experimental manipulation. For example, among the 62 subjects in the sixth group where the effect of the ingestion of 22 caramels was tested, there were 49 individuals caries-free during the control year, 49 caries-free during the ingestion of 345 g. of bread containing sugar, 22 caries-free during the ingestion of 22 caramels and 200 g. of sugar in solution, 19 caries-free on 22 caramels alone and 39 caries-free in the final control year. During the year that 22 caramels were ingested per day, in contrast to the 19 who were caries-free during that year, 12 of the subjects had 4 to 6 new carious areas, another 9 had 7 to 9 new carious lesions, and 2 had 10 or more new lesions. In other words, while the teeth of some individuals had been penalized severely by the incorporation of this amount of candy into the diet, others had been sufficiently resistant that no increase in caries was noted. Similar numbers of caries-free individuals were noted in other groups even though the over-all caries increment for the groups had skyrocketed during the experimental period.

This again points to the individual variation in caries susceptibility which results from various constitutional and developmental variants within the teeth and the individual. The same phenomena have been repeatedly observed in experimental animals. From a genetic standpoint, strains of rodents have been developed which have widely different caries-susceptibilities despite common dietary and environmental circumstances. The long-time studies with the white rat at Michigan State College have clearly demonstrated the ability to develop a caries-resistant and a caries-susceptible strain of rats from common ancestors by a careful selecting and breeding program (W. H. Stewart, C. A. Hoppert, and H. R. Hunt, *J. Dent. Res.* **32,** *210 (1953)*). Likewise, dietary studies during tooth development strongly indicate that the caries-susceptibility of a tooth is to a large extent established during its formation. In rhesus monkeys, for example, the teeth formed in India while the animals are consuming a natural diet remain relatively free from tooth decay for prolonged periods of maintenance on a high sugar diet. In contrast, the teeth of the same experimental monkeys which were formed in captivity during maintenance on purified diets are readily attacked by the carious process upon eruption into the oral environment which has been insufficiently cariogenic to attack the other teeth (R. F. Sognnaes, *J. Calif. Dent. Assn.* **26,** *No. 3, supplementary issue, pp. 37–52, May–June (1950)*).

The Vipeholm data point with vigor to the definitely greater importance of the physical form in which sugars are ingested than the total amount consumed. These data also emphasize the real importance of the several constitutional factors which determine caries-susceptibility and the difficulty with which these factors are overruled by unfavorable oral environmental circumstances. There is a real need for the continued collection of experimental data concerning both oral environmental influences and systemic variants in human beings and laboratory animals in order to outline in detail the varied controlling factors in the etiology of dental caries.

NUTRITION AND BEHAVIOR

The complex interrelationships between nutrition and behavior represent an interesting but underdeveloped sector of the science of human nutrition. An attempt will be made here to bring together several recent studies dealing with different facets of this

problem. Carefully controlled clinical and laboratory investigations, using psychometric and sociometric technics, have been limited largely to the "Western" world. However, it may be that the most important field for a systematic study of the impact of nutrition on psyche lies elsewhere—in Africa, Asia, parts of South America. Thus the presence of mental apathy, combined with peevishness when the patient is disturbed, is cited as one of the outstanding characteristics of kwashiorkor (Joint FAO/WHO Expert Committee on Nutrition, Third Report, *WHO Technical Report Series No. 72, 20 (1953)*).

The literature on mental development in relation to malnutrition has been brought together by J. C. Carothers ("The African Mind in Health and Disease," *World Health Organization Monograph Series No. 17, see esp. p. 110 (1953)*). M. Clark (*E. African Med. J.* **28,** *229 (1951)*) considers the mental changes a consistent and important feature of kwashiorkor. "A child with kwashiorkor is dull, apathetic, and miserable. It rarely cries or screams, a low whimper is the only sign of its wretchedness." J. N. P. Davies (*Ann. Rev. Med.* **3,** *99 (1952)*) notes that a state of peevish apathy dominates the lives of many African children up to the age of 5 years. While his better-fed contemporaries make great strides in actively exploring the world around them, the African child is too often a whining, apathetic invalid. This must be a great handicap to his mental and emotional development. Carothers (*loc. cit.*) believes that arrest of mental development at 2 to 4 years, associated with severe protein deficiency, is unlikely to be wholly remedied in later years. Furthermore, the older child together with the rest of the population is frequently either seasonally or continuously undernourished.

Observations of mental differences between tribes subsisting on different diets are few. In an older survey of two tribes in Northern Rhodesia, A. I. Richards and E. M. Widdowson (*Africa* **9,** *166 (1936)*) noted in the fish-eating Bisa greater vitality, endurance and ability to concentrate than in the Bemba who depended largely on millet for their diet. B. M. Nicol (*J. Clin. Nutrition* **1,** *364 (1953)*) in his study of tribal nutrition and health in Nigeria reported that, on clinical examination, physical fitness and mental alertness were judged satisfactory in about 70 per cent of the trader group and the Ijaw fishermen, while only half as many of the Isoko farmers appeared fit. The majority behaved in an apathetic and uninterested manner while being examined and questioned.

Considerations of their general appearance, behavior and nutritional intake suggested that their mental apathy and irritability were related to long-continued deficiency of B-complex vitamins. A more detailed diagnostic study and, in particular, the evaluation of dietary treatments will require the application of more sophisticated, quantitative technics for the description of mental functioning and of personality. Nicol's study (*loc. cit.*) was limited to clinical impressions, and the records of the local police and the courts providing evidence on the rages and maniacal tantrums from which the Isoko farmers suffer from time to time. The effects of nutrition represent phenomena challenging modern social science by the need for combining the approaches of anthropology, sociology and psychology. The problem of nutrition and psyche can and must be approached from the somatopsychologic point of view (effect of nutrition on behavior), as well as by considering the emotional significance of foods and eating to the individual and to the group.

In France the psychologic aspects of nutrition have been examined in several papers and reviews published by P. Aschkenasy-Lelu. Her experimental study was concerned with spontaneous food intake of the rat, with special reference to the controversial "hunger" for proteins (*Ann. de la Nutrition et de l'Alimentation* **5,** *453 (1951)*). While she accepts the general thesis that metabolic factors play an essential role in

the regulation of the food intake, she has not been able to demonstrate the specific appetite for protein.

Aschkenasy-Lelu's review of literature on nutrition and behavior cites some 80 references (*Encéphale* **40,** *341* (*1951*)). She points out that nutritional deficiencies affect principally the emotional tone and personality while the intellective functions are much more resistant to deterioration. The mode of action may be either direct, when the central nervous system itself is affected, or indirect. Two other publications of Aschkenasy-Lelu deal critically with the effects of "luxus consumption" of certain nutrients, particularly thiamine and glutamic acid (*Ann. de la Nutrition et de l'Alimentation* **3,** *109* (*1949*)), and with nutrition in the treatment of mental disorders (*Encéphale* **41,** *45* (*1952*)).

A. Masson (*Ann. méd.-psychol., 110e année,* **2,** *674* (*1952*)) points out that while mass starvation has been known in recent times in western Europe only in times of wars and in concentration camps, sporadic severe caloric deficits do occur. Consequently, the psychopathology of hunger is of medical and legal interest. The author draws on her psychiatric experience, on literature describing the conditions in the concentration camps, but relies most heavily on Knut Hamsun's autobiographical novel "Hunger" (translated from the Norwegian by George Egerton, *A. A. Knopf, New York* (*1935*)). Some effects of severe inanition, for example, in old people living in isolation, may simulate psychotic symptoms but are reversible by adequate diet.

The psychologic effects of starvation and oral deprivation were examined by J. A. M. Meerloo and L. D. Klauber (*Psychosomatic Med.* **14,** *491* (*1952*)) with special reference to medical and surgical patients in whom parenteral feeding and diet therapy, resulting in drastic changes in food habits, may lead to untoward mental reactions. The senior author had an opportunity to study the human reactions to starvation as an internee in a war camp and while he was attached to a Red Cross feeding team in Holland in 1945. The observations on famine victims are used as the background for the evaluation of clinical starvation which has not been examined systematically.

The case studies are limited to a man fed parenterally, a woman on a rigorous diet, and another woman in whom the symbolic value of food as a source of security was demonstrated. In addition to the somatic factors of the disease, the psychologic effects of food deprivation in patients are frequently complicated by pain and the fears of the original disease, operative procedure and death. Yet it is believed that the absence of oral gratification in starvation may be psychologically important. This depends, to a large measure, on the normal or neurotic emphasis placed on oral gratification by the patient before his illness. In some patients with heart disease put on a rigorous diet there may develop a conflict between the fear of death and the anger associated with the food restriction. Their depression and apathy can only be overcome by providing more variety in foods and more oral gratification. The symptoms which are considered as possible indicators of psychologic changes accompanying food deprivation include the restlessness of postoperative patients, their irritability, and occasional unrealistic and infantile demands.

Here we tread unquestionably on uncertain grounds. The comparisons of the behavior of "postoperative patients" with the symptoms found in the victims of prolonged food deprivation appear questionable if the type, degree and duration of the food restriction are not clearly spelled out. Is it justifiable to interpret the frequent preference of patients for milk and "baby foods" in terms of a regression to infantile dependency? Nevertheless, the problems are real and important, and deserve a good deal more attention by medicopsychiatric and surgicopsychiatric research teams. Patients exposed to semistarvation and oral deprivation because of organic disease or surgical necessity may develop psychologic

reactions which in turn aggravate the disease or paralyze the wish to live. Apathy, depression and paranoid symptoms may complicate the postsurgical care. A compromise with overly restrictive diets may reduce or eliminate these symptoms.

We may accept, in general, the thesis that man's responsiveness to his environment is modified by his physiologic needs. Several investigations have been concerned with the relationship between "hunger" (defined as hours of food deprivation) and perceptual behavior. R. S. Lazarus, H. Yousem, and D. Arenberg (*J. Personality* **21,** *312 (1953)*) reviewed a number of studies on hunger and perception, and carried out additional experiments designed to throw some light on the need-perception relationships. The subjects were tested one, two, three to four, and five to six hours following eating. In the first experiment, 5 photographs of common food objects and an equal number of other objects were presented to the subjects individually for a very brief period (0.2 seconds) while the intensity of illumination was increased in small steps.

The number of trials needed to perceive correctly the food pictures indicated a high threshold at 0 to one hour of food deprivation, a progressive decrease after three and four hours, with a sharp rise after five to six hours (17.0, 15.3, 14.9 and 16.6 trials). The difference between the mean scores for the food and nonfood pictures followed a similar pattern. The authors concluded that the threshold of recognition for the food items varied significantly with the degree of food deprivation and subjective hunger. The fact that the average scores for the longest period of going without food, associated with highest rating of subjective experience of hunger, did not follow the initial pattern is not adequately explained. The authors believe that the "need" operates in terms of the cycle of eating, decreasing when the subject normally expects to eat; but it has been noted above that the subjective hunger was highest at this time, with ratings of 1.9, 2.4, 3.0 and 3.3 for the four periods. In the word association test, given at the same testing sessions, there was no tendency for the hungrier subjects to give more food responses. This finding was in line with the negative data obtained in a prolonged semistarvation studied by J. Brozek, H. Guetzkow, and M. V. Baldwin (*J. Personality* **19,** *245 (1951)*).

From the review of the studies cited above it is clear that the relations among diet, emotions and behavior are complex, including food habits, symbolic values attached to food and eating, and motivated perception, as well as changes in physical fitness, mental alertness and personality which result from deficiencies of certain nutrients. The practical implications of these facts would lead one to expect more intensive research in this field that can be actually documented. The obstacles are as large as many of the problems are important. While reasonably satisfactory tools for a quantitative or at least semiquantitative description of intelligence and personality are available for use in the laboratories and clinics of English-speaking countries, these instruments in the overwhelming majority are wholly inapplicable to native populations. However, progress in the scientific study of the gross and the more subtle impacts of diet on man's mind and behavior is one of the most intriguing developments in a comprehensive science of nutrition.

RECOMMENDED DIETARY ALLOWANCES—1953

A revision of the Recommended Dietary Allowances of the Food and Nutrition Board has appeared (*National Research Council Publication No. 302 (1953)*). In general the allowances confirm the previous recommendations (*National Research Council, Reprint and Circular Series No. 129, Washington (1948)*), but several important changes have

been made. The most obvious changes are in the size of the standard man and woman which are now given as 65 and 55 kg. respectively rather than 70 and 60 kg. as formerly, the basis and method of presentation of calorie allowances with consequent changes in the recommendations of those nutrients which are calorie-dependent, the division of the first year of life into four age groups, and a lowering of the calcium allowance of adults from 1.0 g. per day to 0.8 g. per day.

The new calorie allowances are similar to those presented by the Food and Agriculture Organization of the United Nations ("Calorie Requirements," *FAO Nutritional Studies No. 5, Washington (1950)*). "The basis of the recommendations is the calorie allowance for a 'standard' man or woman, both aged 25, living in a temperate climate (mean annual temperature 10°C.) and weighing 65 and 55 kg. respectively. These standard persons are assumed to be fairly active physically. . . ."

The recommended allowances are 3200 and 2300 calories respectively. Adjustments to be made for age, body size, climate and activity are described. Since the table now presents three age groups, 25, 45 and 65 years, with the calories adjusted downward by 5 per cent per decade over age 25, rather than values for three levels of activity as in the earlier table, it was apparently the committee's opinion that this method of presentation was more useful than the earlier one. Although the outstanding factor causing variability in calorie needs is physical activity, it is pointed out that no simple method to evaluate this is available. Most dietary surveys in the past have simply reported adults as physically active; we may expect that surveys in the future will take into account the general decrease in activity with age and the associated fall in dietary requirements. Similarly, the directions for adjustment for body size and climate may encourage more detailed description of the subject material.

In the previous recommendations for infants under one year of age the values for protein and calories were based upon weight, and a fixed value for the other nutrients was given. The new recommendations recognize the rapid rate of growth and development during this age period. No recommendations are given for the first month of life since "desirable allowances for many nutrients are dependent upon maturation of excretory and endocrine functions." Allowances for all nutrients gradually increase for the three age groups specified during the first year, thus eliminating at least in part the previous condition where the allowances were either too high or too low for most of the infants under one year of age. The low protein and calcium contents of human milk present difficulties and the allowances are said to apply to nutrients derived primarily from cow's milk. "If the milk from which the protein is derived is human milk or has been treated to render it more digestible, the allowance may be in the range of 2–3 gms. per kg. There should be no question that human milk is a desirable source of nutrients for infants even though it may not provide the levels recommended for certain nutrients."

It is of considerable interest that the recommendation for calcium for adults has been returned to the earlier level of 0.8 g. per day. The report states that this decision "was made after due consideration of the available balance data and of metabolic and dietary factors which pertain to the utilization of calcium. The basic assumption for this recommendation was that the adult calcium allowance (except for pregnancy and lactation) should provide for calcium equilibrium throughout adulthood but the desirability of continued calcium storage after completion of growth is a matter of conjecture."

Although riboflavin allowances remain approximately the same as in the previous table, related to weight rather than calories, they have now been made proportional to the protein allowance which is also based upon weight. Multiplying the protein allowance by the factor 0.025 gives the riboflavin allow-

ance in milligrams. This is simply a matter of convenience and implies no specific relation to protein metabolism. The riboflavin allowances for pregnancy and lactation are 0.5 mg. per day less than previously given.

Although the ascorbic acid allowance of the British (British Medical Association, Report of the Committee on Nutrition, *London* (*1950*)), and Canadian (Canadian Council on Nutrition, *Canad. Bull. Nutrition* **2,** *6* (*1950*)) advisory groups is given as 30 mg. for the adult, the revised NRC allowances adhere to the earlier values, 75 mg. for adult man. It is stated that the "values given in the table represent an appraisal of all the evidence available. They are not saturation values since more generous intakes result in distinctly higher concentrations in the tissues. The recommended allowances do permit tissue storage within the range found to be approximately normal for the tissues of animals in which the vitamin is under physiological control, and in human tissues when there is a nominal intake of antiscorbutic foods."

It is well known that the recommended allowances have been criticized from time to time, principally for being unduly generous. Anyone contemplating the use of these allowances should be instructed to read the entire publication. The philosophy of the allowances and their scientific basis is clearly presented. In particular it is stated that "The allowances are designed for the maintenance of good nutrition of healthy persons in the United States under present conditions. They are not necessarily applicable to situations of stringency or limited food supply. The recommendations are not requirements, since they represent not merely minimal needs of average persons, but nutrient levels selected to cover individual variations in a substantial majority of the population. In addition the values for each nutrient above the minimal level which will prevent dietary deficiency are considered to provide for increased needs in times of stress and to permit other potential benefits. Although the optimal intake of essential dietary constituents remains largely speculative, there is considerable evidence that improvement in growth and function occurs when the intake of certain nutrients is increased above the level just sufficient to prevent deficiency disease. The nutritive intakes recommended are, in general, higher than average requirements and lower than the amounts which may be needed in pathologic states or in rehabilitation following depletion." In contrast to this philosophy the British standards "are believed to be sufficient to establish and maintain a good nutritional state in representative individuals of population groups." The Canadian standards provide "a nutritional floor beneath which the maintenance of health of the people cannot be assumed." The relatively generous nature of the present allowances is thus clearly recognized. Since the value of an allowance for safety must remain speculative for the present, there is room for argument and discussion, but an understanding of the approach used by the committee in establishing the recommendations would prevent considerable misuse and some unjust criticism that has occurred in the past.

The Recommended Allowances provide a basic tool and the standard of reference for all concerned with nutrition.

LOW FAT DIETS IN LAMBS, GOATS AND CALVES

Fat deficiency has been produced in mice, dogs, chickens and pigs since the original observation by G. O. Burr and M. M. Burr (*J. Biol. Chem.* **82,** *345* (*1929*)) that fat was essential in the diet of rats. Animal nutritionists have questioned in recent years whether the more extensive processing of soybeans, cottonseed and peanuts by the

solvent method might result in a tendency toward critically low fat levels in the feed of cattle. Hydraulic or press methods of removing oil from the seeds of these crops generally result in a pressed cake or oil meal containing up to 5 or 6 per cent of fat, whereas solvent extraction procedures generally reduce the oil meal to fat levels of less than 2 per cent. With more and more solvent-extracted seed meals appearing on the market, the question of the essentiality of fat in the nutrition of ruminants has recently received attention at Cornell University and at Iowa State College.

At Cornell, H. M. Cunningham and J. K. Loosli (*J. Animal Sci.* **13,** *265 (1954)*) investigated the effect of fat-free diets on lambs and goats. Two experiments were conducted. In the first, 6 weanling lambs were gradually changed from a ration of good alfalfa hay and oats to a purified diet of cerelose, cellophane, alpha-protein, minerals and vitamin supplement. After one hundred twelve days the alpha-protein was replaced by a nitrogen equivalent of urea to insure a completely fat-free diet. Two of the 6 lambs served as controls and received corn oil in the diet at a level of 1.5 per cent.

Rumen contents as well as blood samples were analyzed at varying intervals for total fat and for linoleic, linolenic and arachidonic acids (the fatty acids that are effective in curing fat deficiency in rats). The lambs receiving corn oil had significantly higher blood levels of total fat (138.5 mg. per cent) than the animals receiving the fat-free diet (102.5 mg. per cent). The linoleic acid content of blood from fat-supplemented lambs averaged 17.4 mg. per cent and this was significantly higher than the level of 7.9 mg. per cent in the blood of deficient lambs. Rumen contents of the lambs on the fat-deficient diet contained only traces of linoleic and linolenic acids, and no arachidonic acid was detected. Isolated rumen bacterial cultures were grown on fat-free mediums. These were noted to synthesize appreciable quantities of fat but did not produce any detectable amounts of the essential fatty acids. Although the lambs on the fat-deficient diet failed to develop any gross symptoms suggestive of fat deficiency, it was concluded that the experimental diet was unsatisfactory for the demonstration of deficiency symptoms because the lambs failed to gain weight even with added dietary fat.

In experiment two, 2-day-old lambs and 2-day-old goats were used and the diet was a synthetic milk containing about 13 per cent of solids in the form of alpha-protein, methionine, cerelose, minerals and vitamins. After two weeks a mixture of shredded cellophane, alpha-protein, glucose, potato starch and minerals was fed ad libitum as a supplement to the synthetic milk. Lambs receiving this diet and a supplement of 25 g. of lard per kilogram of synthetic milk (the approximate amount consumed daily per lamb) gained in weight about ¼ pound daily during the ten-week experimental period. On the other hand, the lambs receiving the fat-free diet showed only slight weight gains for a few weeks and then developed a fat-deficiency syndrome that ended fatally. The syndrome was characterized by extreme weakness, listlessness, retention of large amounts of fluid in the abdominal cavity, and a continual chewing movement of the jaws. Lambs receiving the lard supplement had higher blood plasma levels of total fat, arachidonic and linoleic acid than the deficient animals after a four-week experimental period.

Fat deficiency in the calf was investigated by workers at Iowa State College (M. R. Lambert, N. L. Jacobson, R. S. Allen, and J. H. Zaletel, *J. Nutrition* **52,** *259 (1954)*). Calves fed a semisynthetic diet containing 3.5 per cent of casein, 5.0 per cent of lactose, 0.2 per cent of salts and variable small amounts of lecithin gained weight at a very slow rate (10.9 pounds in fifty-six days). In comparison, calves fed the same diet plus 1.8 per cent of hydrogenated soybean oil and 0.2 per cent of lecithin gained 30.3 pounds in the same period. Other symptoms of fat

deficiency were scaly dandruff over much of the body surface, lack of luster in the hair coat, and loss of hair on the neck and tail. Diarrhea occurred in the low-fat as well as the fat-supplemented animals but was much more severe among the fat-deficient calves. After twelve weeks on the low-fat diet, fats were introduced into the diet with resultant recovery from the deficiency symptoms. Butter oil, methyl esters of oleic and linoleic acids, and hydrogenated soybean oil plus lecithin were all effective in relieving the deficiency symptoms.

In most instances the low-fat diet resulted in decreased blood plasma levels of total fat and unsaturated fatty acids. Linolenic acid levels were low (less than 5 mg. per 100 ml. of plasma) in all calves regardless of diet. As was found in the Cornell study with lambs and goats (*loc. cit.*), the fatty acid that decreased in the blood plasma to the greatest extent on fat-deficient diets was linoleic acid. This acid was present in blood plasma at a level of about 25 mg. per cent after eight weeks of fat-deficient diet as contrasted with a level of 80 mg. per cent in the plasma of calves receiving fat in the diet.

What these studies on the fat requirement of ruminants may contribute to our knowledge of fats in human nutrition may seem obscure. However, these studies with farm animals confirm recent observations on dogs that blood serum levels of the unsaturated fatty acids reflect the nutritional status of the animal with respect to amount and kind of fat in the diet.

On the basis of these findings, workers at the University of Texas have recently investigated the serum level of unsaturated fatty acids in healthy and in poorly nourished infants and children (H. F. Wiese, R. H. Gibbs, and A. E. Hansen, *J. Nutrition* **52,** *355 (1954)*; Hansen and Wiese, *Ibid.* **52,** *367 (1954)*). The serum of healthy infants was lower in linoleic and linolenic acids than that of children 2 to 15 years of age. On an intake of linoleic acid of about 3 per cent of the diet the mean serum levels of linoleic, linolenic, and arachidonic acids (2, 3, and 4 double-bond fatty acids) for 60 healthy children aged 4 to 15 years were 30.3, 1.5, and 10.2 per cent, respectively, of the total fatty acids. Since the diet contained only traces of arachidonic acid and this acid comprised 10 per cent of the serum fatty acids these workers assumed that there is no dietary requirement for arachidonic acid when the diet contains linoleic acid. It should be pointed out that on the basis of animal experiments it is fairly well established that the active essential fatty acid for animals is arachidonic and that linoleic acid is converted to this more active compound in the animal. These workers found poorly nourished children to have significantly lower serum levels of linoleic and linolenic acids than normal children, whereas the total serum fatty acids were not reduced in the poorly nourished group.

Considerably more studies are obviously needed before the significance of these results can be interpreted in terms of nutritional requirements for fat by human subjects and also in terms of specific fatty acid requirements. The results, however, are indicative that fractionation of serum fatty acids may serve as another tool in the evaluation of nutritional status.

ANTIBIOTICS AND THE INTESTINAL FLORA

A number of recently published papers have called attention to the growth-promoting properties of certain antibiotics such as aureomycin and penicillin. These findings together with those from other types of experiments have considerably altered the current concepts of the role of the intestinal flora in the nutrition of the host animal. Sulfa drugs were introduced into nutritional research in an effort to reduce the

bacterial population of the gastrointestinal tracts of experimental animals. These efforts aimed at reducing the rate of synthesis of vitamins of the B-complex by the intestinal microorganisms. It was the use of sulfa drugs which permitted the clear demonstration of deficiencies in biotin and folacin in rats and mice. These species, and some others, are able to exist for considerable periods of time without dietary biotin and folacin since these vitamins are synthesized and released by intestinal bacteria at rates sufficient to meet the nutritional needs of these animals.

Interestingly enough, administration of the sulfa drugs decreased the bacterial population only temporarily. The total counts of organisms usually returned to normal, although the synthesis of vitamins continued to be depressed. This depression was attributed to the proliferation of a sulfa-resistant flora which did not possess vitamin-synthesizing properties, or exhibited these properties to a significantly smaller degree.

Not long ago it was demonstrated that some of the newer antibiotics could, under certain conditions, stimulate growth. These observations indicated that the antibiotics suppressed those components of the intestinal flora which normally deprive the host of significant quantities of ingested nutrients. Alternatively the antibiotics, by suppressing some members of the gastrointestinal population, might permit the more extensive proliferation of organisms which contribute materially to the nutrition of the host. Several investigators have shown that the growth-stimulating effect of antibiotics is due to increased food intake; the effect is not observed when the animals receiving the antibiotic are restricted in food intake to the level of animals not receiving the drug. There are many indications that the increase in food intake with antibiotic administration is related to the changes in the intestinal flora.

A study attempting to correlate the ability of penicillin to increase food consumption and growth in chicks with the drug's effect on the intestinal flora has been reported by S. J. Slinger, M. M. Hauser, and W. F. Pepper (*J. Nutrition* **52,** *75 (1954)*). Day-old chicks were fed a stock ration with or without penicillin for a period of twenty-eight days. Some animals receiving penicillin were limited in food intake to the quantity consumed by controls fed the unsupplemented basal ration. Other penicillin-receiving chicks were fed ad libitum. Body weights, food consumption and fecal flora were checked daily. After the initial twenty-eight-day period all animals were continued for an additional three weeks with ad libitum feeding. The feces were cultured for coliform bacilli, lactobacilli, aerobic organisms, anaerobes, and enterococci. Apparently no total counts were made.

When the rations were fed ad libitum, the addition of penicillin increased body weight. Under restricted feeding penicillin had no significant effect. The drug had no effect on the efficiency of food utilization for growth but increased the amount of food voluntarily consumed. Daily coliform counts gave erratic results for the first seven days of the experiment. During this period the characteristic intestinal flora becomes established out of drastic changes in populations as different types of microorganisms compete for survival or dominance. After this initial period the coliform counts of the penicillin-treated animals were higher than those of the controls. Near the end of the twenty-eight-day period the coliform counts of the two groups of chicks reached approximately the same level.

In both groups the coliform bacteria increased slowly during the experiment. On the other hand, the enterococci diminished with age, decreasing somewhat more slowly in the animals receiving penicillin. The authors report the drug to cause a transient decrease in lactobacilli count under conditions of ad libitum feeding. Examination

of the data makes it difficult to agree with this conclusion.

Highly significant positive correlations were found between daily measurement of food consumption (with or without penicillin) and daily coliform counts. Equally significant negative correlations were noted between food intake (without drug) and enterococci counts. There was no correlation between food intake and lactobacilli, aerobe, or anaerobe counts. The authors suggest the existence of a causal relationship between the increase in coliform bacteria and the increase in food consumption. They also suggest that enterococci may lower food intake.

It is unfortunate that attempts to characterize the intestinal flora, and to detect changes in the flora, suffer from the cumbersome technics of culturing. Although Slinger *et al.* used five different culture mediums (a major undertaking), the fecal flora has been characterized only with respect to five broad and overlapping groups of organisms. The correlation between coliform count and food intake is striking. But which members of the coliform group—*Escherichia coli*, *E. intermedium*, *Aerobacter aerogenes*—are actually responsible for this relationship? Which of the enterococci depress food intake? Obviously, present methods of culturing make the quantitative estimation of genera, species and strains nearly impossible if more than a few animals are to be used in an experiment. Although research in methodology is not very popular, there is an urgent need to develop comprehensive yet simple culturing methods.

The effect of aureomycin on the intestinal synthesis of vitamin B_{12} has been studied by K. R. Johansson, G. E. Peterson, and E. C. Dick (*J. Nutrition* **49,** *135* (*1953*)) in an attempt to explain the growth-promoting effect of the antibiotic. The study was carried out on rats fed either a synthetic diet low in methionine and vitamin B_{12} or a stock ration presumably adequate in all essentials. Feces were cultured in nine different mediums and were analyzed for vitamin B_{12}.

Aureomycin stimulated the growth of rats when it was added to the synthetic diet fortified with vitamin B_{12}. Addition of the antibiotic to the stock ration was without effect. This is in contrast to the findings of Slinger *et al.* (*loc. cit.*). The drug had no clear effect on the amount of "free" vitamin B_{12} present in the feces. Aureomycin added to either the synthetic or the stock ration caused significant changes in the fecal flora with the exception of the counts of coliform bacteria and the total number of organisms from animals receiving the synthetic diet. The number of lactobacilli was decreased and the enterococci increased. Since lactobacilli require many preformed nutrients, a reduction in their number is presumably advantageous to the host.

These same workers (G. E. Peterson, E. C. Dick, and K. R. Johansson, *J. Nutrition* **51,** *171* (*1953*)) studied the effect of aureomycin fed in conjunction with a sucrose- or dextrin-containing diet. Addition of this antibiotic to the dextrin-containing diet resulted in a marked increase in coliform bacteria, while its addition to the sucrose diet did not affect this group of organisms. On the other hand, J. Guzman-Garcia, W. B. Sarles, and C. A. Baumann (*Ibid.* **49,** *647* (*1953*)) reported increases in intestinal coliform bacilli when penicillin was added to a high-sucrose diet. Since Peterson and co-workers chose to express organism counts per gram of material cultured, the data furnished may not tell the entire story. The authors themselves call attention to the fact that the cecal contents were increased by aureomycin feeding. It seems likely that the drug may cause small changes in the counts of certain organisms per gram of cecal contents but actually cause much larger changes in the number of such organisms present in the entire cecum. The "free" vitamin B_{12} in the intestinal contents was increased by aureomycin feeding and there was high correlation

between "free" vitamin B_{12} and growth response.

While there are some differences between the results of Peterson *et al.* and those of Slinger *et al.*, there are also many points of agreement. Both reported growth stimulation by antibiotics under certain conditions. Both found some increase in coliforms. Peterson and co-workers were able to correlate intestinal vitamin B_{12} "synthesis" with growth response. Slinger *et al.* correlated coliform counts with food intake.

THE METABOLISM OF LYSINE

The metabolic fate of lysine in the body presents certain interesting peculiarities. The essentiality of this amino acid in animal nutrition was demonstrated by Osborne and Mendel in experiments in which the dietary protein was provided by the vegetable protein, gliadin. With the advent of the use of isotopic tracers in the study of intermediate metabolism the unique behavior of this nutrient within the body became apparent. Lysine is much less reactive than other amino acids. In rats fed amino acids labeled with N^{15}, Schoenheimer found that lysine was the only amino acid which invariably failed to take up N^{15}. When lysine labeled with both deuterium and N^{15} was fed to animals the ratio of the two isotopes in the lysine isolated from the tissue proteins was found to be the same as the administered lysine. As might be predicted from the above experiments the alpha-keto analogue of lysine was found to be nutritionally inactive and the organism is unable to invert the D-isomer into the nutritionally active L-isomer.

Oxidative deamination of lysine does however take place within the body and is irreversible. The fate of the carbon skeleton of lysine has been the subject of a recent report by L. L. Miller and W. F. Bale (*Arch. Biochem. Biophys.* **48,** *361 (1954)*). Using a previously described method, D,L-lysine was labeled with C^{14} in the sixth carbon atom, the carbon atom bearing the terminal amino group. Two g. of this material was fed to 2 fasted dogs. The first dog received in addition 180 g. of chopped beef and the second dog received 150 g. of chopped beef. After twenty-four and twenty-seven hours respectively the dogs were drained of blood under ether anesthesia. The dogs were then perfused to render the organs essentially bloodless.

Lysine, glutamic acid, aspartic acid and arginine were isolated from hydrolysates of liver and plasma proteins using chromatographic technics. The activity of the isolated lysine in disintegrations per minute per milligram $\times 10^{-4}$ was 4.49 for liver protein and 5.28 for plasma protein in the first experiment. Radioactivity was present in the other three amino acids. The activity of glutamic acid was slightly greater (0.75 and 0.61 for liver and plasma protein respectively) than in aspartic acid and arginine where the values for both liver and plasma proteins were in all cases close to 0.3. The results in the second experiment were comparable. Because of the similarity of the radioactivity of these three amino acids it was suspected that they had a common precursor.

To study further the mechanism of incorporation of the sixth carbon of lysine into glutamic acid the isolated glutamic acid was degraded by both the Schmidt reaction and the ninhydrin reaction. The Schmidt degradation removes the terminal carboxyl of glutamic acid which would correspond to the sixth carbon of lysine. In both liver and plasma glutamic acid this carbon contained about three quarters of the activity. One quarter of the original activity was found in the decarboxylated residue. The ninhydrin reaction removes the alpha carboxyl, and this carbon was found

to contain one quarter of the radioactivity. When the ninhydrin degradation was applied to aspartic acid isolated from liver and plasma proteins the radioactivity was found in the liberated carbon. In the case of aspartic acid, however, both carboxyl groups are removed so that the findings are still consistent with the hypothesis that aspartic and glutamic acids arise from the same precursor.

In discussing these results the authors present arguments that the incorporation of carbon from lysine could not all occur by way of small fragments, but that the incorporation of the carbon skeleton of lysine was likely. It was suggested that glutaric acid might be the real intermediate in the metabolism of lysine on the basis of unpublished findings that glutaric acid-1,5 C^{14} is metabolized as rapidly as L-lysine and is converted to ketoglutarate in addition to acetoacetate and acetate. In summary, two pathways are available for the incorporation of carbon from lysine to form glutamic and aspartic acids, the first by a direct conversion to ketoglutarate, perhaps via glutarate, and the second by an indirect path via acetate.

When the arginine isolated in these experiments was degraded to ornithine it was found that this fragment contained a considerable portion of the radioactivity. The pathway of conversion of the carbon chain of lysine to arginine cannot be determined on the basis of the present data. The possibility that glutamic acid might be in the path of incorporation is suggested by the findings of M. Womack and W. C. Rose (*J. Biol. Chem.* **171,** *37 (1947)*) who were able to correct a relative growth-limiting deficiency of arginine in the diet of rats by the addition of glutamic acid.

From these experiments we may conclude that one of the metabolic pathways in the metabolism of lysine is the incorporation of the carbon skeleton into other amino acids possessing five or six carbon atoms in an unbranched chain.

PORPHYRIN SYNTHESIS

The nutritionist has been interested in the mechanism by which hemoglobin is synthesized, because anemia is a common finding in a number of deficiency diseases. Hemoglobin is a chromoprotein consisting of the protein globin linked with the pigment heme. The latter consists of protoporphyrin and iron. Hemoglobin synthesis may be impaired by interference with the synthesis of globin from its component amino acids, by reduction of protoporphyrin synthesis, or by reduction of available iron for incorporation into the heme pigment. Such impairment may be brought about either by reduction of the quantities of available building materials, or by interference with the chemical mechanisms effecting incorporation of these materials into the finished products. Two recent papers have dealt with certain aspects of protoporphyrin synthesis. J. Lucas and J. M. Orten (*J. Nutrition* **52,** *89 (1954)*) have investigated in further detail the role of glycine in protoporphyrin synthesis, while E. I. B. Dresel and J. E. Falk (*Biochem. J.* **56,** *156 (1954)*) have made a beginning in the analysis of the mechanism of protoporphyrin synthesis.

J. M. Orten and J. M. Keller (*J. Biol. Chem.* **165,** *163 (1946)*) studied the effect of altering dietary protein intake on the formation of protoporphyrin. They found that low protein intake resulted in decreased protoporphyrin formation. A similar observation was made by J. R. Totter, E. S. Amos, and C. K. Keith (*Ibid.* **178,** *847 (1949)*). These workers also demonstrated that the addition of sodium benzoate to the ration interfered with the manufacture of the porphyrin. It has long been known that sodium benzoate administered to an experimental animal is conjugated with the amino acid glycine and

subsequently excreted in the urine as hippuric acid. This suggested that the benzoate interfered with protoporphyrin synthesis by depleting the animal of available glycine. The inhibitory effect of sodium benzoate was counteracted by the introduction of additional glycine in the experimental ration. These studies showed that glycine plays a significant role in the synthesis of protoporphyrin. A more direct demonstration of the role of glycine came from the studies of D. Shemin and J. Wittenberg (*Ibid.* **192,** *315 (1951)*), who found that isotopically labeled glycine and acetate were incorporated into the protoporphyrin molecule.

In the recent study by Lucas and Orten (*loc. cit.*), adult male rats were subjected to the following dietary regimens: (1) a basal ration containing 20 per cent casein; (2) the basal ration plus 2 per cent glycine; (3) the basal diet with 3 per cent sodium benzoate added (increased to 10 per cent sodium benzoate after four weeks); (4) the basal diet plus 3 per cent sodium benzoate and 2 per cent glycine (changed after four weeks to 10 per cent sodium benzoate and 5 per cent glycine); and (5) a low protein ration. All experimental groups were maintained on one ration for a period of four weeks and thereafter transferred to another diet for an additional four-week interval. The amount of porphyrin excreted in the feces was used as an index of protoporphyrin synthesis by the experimental animal. Feces were collected twice weekly and analyzed for total porphyrin, coproporphyrin and uroporphyrin. However, in these experiments only traces of coproporphyrin and uroporphyrin were detected.

When the experimental animals were fed the basal ration, porphyrin excretion was relatively constant at approximately 30 micrograms per day per 100 g. of body weight. The rate of excretion was increased slightly by the addition of glycine to the basal diet, although in the absence of statistical analysis it cannot be said whether this increase is significant. The addition of sodium benzoate at the 3 per cent level decreased the protoporphyrin excretion. The change to a 10 per cent sodium benzoate level markedly depressed the porphyrin output. It also resulted in the death of 11 out of 12 experimental animals. Supplementation with glycine reversed the sodium benzoate inhibition and porphyrin excretion returned essentially to normal. The feeding of a low protein diet depressed porphyrin excretion. The authors also calculated protoporphyrin excretion in terms of the available glycine. This showed that the small amount of glycine available to animals on the low protein ration was very efficiently utilized for protoporphyrin synthesis and that this synthetic pathway has apparently a high priority when a limited quantity of glycine is available to the organism.

An interesting system for the study of the mechanism of synthesis of blood pigments has been reported by Dresel and Falk (*loc. cit.*). They found that hemolysed erythrocytes of chicken blood contained all the enzymes and other substances necessary for incorporating labeled glycine into the heme portion of hemoglobin. The hemolysate was prepared by centrifuging the blood and washing the cells with 0.9 per cent sodium chloride at low temperatures. The washed cells were treated with water at 0°C. in order to rupture the cell membranes and release the cell contents. The resulting hemolysate was mixed with phosphate buffer and potassium chloride in order to reconstitute an approximately isotonic solution. This preparation was centrifuged to remove cell fragments and small granules. Analyses were made for total protoporphyrin (derived under these conditions mainly from the heme portion of hemoglobin). In addition, attempts were made to determine the concentration of free coproporphyrin and free protoporphyrin always present in the hemolysate.

When the hemolysate was incubated with isotope-labeled glycine, magnesium chloride, sodium succinate, sodium acetate and

adenosine monophosphate, significant incorporation of glycine into the isolated protoporphyrin could be demonstrated. Incorporation was virtually linear for the first two hours of incubation and thereafter fell off until it reached minimal rates after about eight hours of incubation. The addition of a boiled yeast extract considerably enhanced the rate of incorporation of glycine into the isolated protoporphyrin. The factors responsible for this effect of yeast extract have not been identified.

Studies of the effects of variations in the preparation of hemolysates showed that permitting white blood cells to remain in the material being hemolysed decreased the glycine incorporation by 30 per cent. Storage of the hemolysate for twenty-four hours at low temperatures resulted in only a slight loss of activity. The method employed for bringing the hemolysate to approximately isotonicity had no appreciable effect on the activity of the preparation. The inclusion of phosphate buffer was also of no benefit. Energy-yielding co-substrates such as succinate, alpha-ketoglutarate and acetate, inorganic salts like magnesium chloride and ferrous sulfate, and co-factors such as diphosphopyridine nucleotide and adenosine monophosphate exerted no appreciable effect when added to the incubation mixture. On the other hand, adenosine triphosphate, the ubiquitous energy donor, stimulated glycine uptake by approximately 30 per cent. The metabolic inhibitors dinitrophenol and sodium fluoride had an inhibitory effect on glycine incorporation. Calcium chloride also inhibited heme synthesis.

Dresel and Falk also studied the effects of hemopoietic factors on the ability of blood hemolysates to incorporate glycine into protoporphyrin. The inclusion of vitamin B_{12} in the incubation mixture had no demonstrable effect on glycine uptake. Similarly, hemolysates prepared from the blood of vitamin B_{12}-deficient chicks were as active as preparations made from normal animals. The addition of folacin in concentration of 1 mg. per milliliter of incubation mixture stimulated glycine incorporation into protoporphyrin. The addition of ascorbic acid also stimulated heme synthesis but did not enhance the effect of folacin. Folinic acid was ineffective. The addition of aminopterin as a folacin antagonist stimulated glycine uptake almost as much as did folacin. When both were used simultaneously only slight stimulation of heme synthesis was noticed. In subsequent experiments, however, the use of aminopterin alone caused a measurable inhibition of glycine incorporation into protoporphyrin.

Further *in vitro* studies with preparations such as red cell hemolysates might contribute much to an understanding of the chemical mechanisms by which glycine and acetate form protoporphyrin and the locus of action of the several vitamins which influence hemoglobin synthesis.

EFFECTS OF ANTIMETABOLITES OF FOLACIN ON CELL DIVISION

A variety of experimental observations has demonstrated that folacin promotes the growth of animals and is important to cell proliferation. Antimetabolites of the vitamin inhibit the growth of experimental animals, tumors and bacteria (*Nutrition Reviews* **8,** *282 (1950)*; **9,** *24 (1951)*; **10,** *84 (1952)*). The mechanisms involved in this inhibition have not been ascertained, but it has been shown that citrovorum factor is far more effective in combating the effects of the antimetabolites than is folacin itself (*Ibid.* **11,** *348 (1953)*). Other evidence has indicated that certain immature cells, like those in acute leukemia, may be more susceptible to deficiency in folacin induced by biologic antagonism than are mature cells (S. Farber, *Blood* **7,** *107 (1952)*).

A recent report is concerned with the effects of antimetabolites of folacin on cell division and demonstrates an arrest of mitosis (W. Jacobson, *J. Physiol.* **123,** *603 (1954)*). These were cytologic studies which utilized a technic of fixing and staining tissues which was described in an earlier publication (Jacobson and M. Webb, *Exp. Cell Res.* **3,** *163 (1952)*). The staining procedure is claimed to permit differentiation of of desoxyribonucleic acid and ribonucleic acid in dividing cells.

In determining the effect of folacin antimetabolites on cell division it was necessary to examine relatively large numbers of nucleated cells in mitosis so that the distribution of cells in the four recognized stages of mitosis might be effectively ascertained. It should be recalled that in the earliest phase, prophase, there is separation of the nuclear mass into chromosomal threads, and the nuclear membrane and nucleolus persist. In the second stage, metaphase, the nuclear membrane and nucleolus disappear and chromosomes are oriented as a central mass in the cytoplasm. There follows separation of the two sets of chromosomes with beginning formation of the daughter cells, anaphase. Finally, in telophase the nucleus begins to take on the characteristics of the resting nucleus complete with nuclear membrane and nucleolus, and the cytoplasm is divided, with formation of two distinct cells.

The author utilized cells from a variety of sources in his investigations. The bone marrow of 12 patients with acute leukemia was studied. In one patient given 5 mg. of A-methopterin (4-amino, 10-methylpteroylglutamic acid) by injection, there was an accumulation of bone marrow cells in metaphase twenty-four hours after injection. Comparative percentages of dividing cells in metaphase were 45 and 60 per cent respectively before and after the injection of the antagonist. Results are stated to have been similar in the other patients and there was, in addition, a substantial reduction of cells in the later phases of mitosis.

These observations were extended with studies of the bone marrow of mice with acute leukemia. In this instance it was possible to administer relatively larger quantities of folacin antagonist. Actually, 1 mg. of aminopterin was given by subcutaneous injection and the bone marrow was examined after two hours. In a representative experiment 70 per cent of dividing cells were in metaphase as compared with 48 per cent prior to injection. The proportion of cells in prophase was unchanged and very few cells could be found in anaphase and telophase following aminopterin administration.

To evaluate the effect of the folacin antagonist on cell division in other tissues, the epithelium of the small intestine of the mouse was utilized. It was found that within two hours following injection of 0.2 mg. of aminopterin there was accumulation of cells in metaphase and clumping of chromosomes. After three days there was evidence of failure to replace epithelial cells, with flattening of the epithelial lining of the glands and contraction of the intestinal villi. Often the epithelium broke down, leaving defects in the mucosa.

Another approach was utilized in the further investigation of this phenomenon. This was an *in vitro* study of the effect of aminopterin added to actively growing tissue cultures of fibroblasts and osteoblasts taken from the frontal bone of chick embryos. When a solution of 1:2000 aminopterin in Tyrode solution was added to a tissue culture, there were marked changes in distribution of the mitotic stages. In this instance, also, there was accumulation of cells in metaphase, and the number of cells in anaphase was reduced greatly. The illustrations which accompany the paper demonstrate clumping of chromosomes and absence of the characteristic metaphase plate of chromosomes. It was found that this inhibition and arrest of mitosis was released with longer contact of the antagonist with the cells, that is, for periods of from twenty-four

to seventy-two hours. Smaller concentrations of aminopterin in tissue culture mediums produced similar effects on mitosis. However, no inhibition was apparent with concentrations of the antimetabolite less than 1:50,000.

The observations described are consistent in demonstrating an arrest of mitosis in metaphase. There seemed to be no interference with the initiation of cell division, and those cells which had progressed to the stage of anaphase prior to the influence of aminopterin preserved their ability to complete mitosis.

The author in further work (W. Jacobson, *J. Physiol.* **123,** *618 (1954)*) has attempted to overcome the mitotic arrest induced by aminopterin by using folacin and citrovorum factor. The addition of folacin in relatively great concentration as compared to the aminopterin added to tissue cultures failed to affect the mitotic arrest induced by the antagonist. This was true both when the vitamin was added simultaneously with aminopterin or one hour prior. In contrast, citrovorum factor protected against mitotic arrest when added at the same time as aminopterin. Increasing concentrations of the antimetabolite required relatively greater concentrations of citrovorum factor to prevent arrest in metaphase.

The failure of folacin to interfere with this action of aminopterin suggests that the antagonist does not replace folacin in the metabolism of dividing cells and that the fibroblasts and osteoblasts of the tissue culture do not convert the vitamin to a compound which competes metabolically with aminopterin. On the other hand, the conclusion seems warranted that citrovorum factor is important to the normal progress of mitosis, and arrest in metaphase occurs because aminopterin replaces it or a related compound in the metabolism of dividing cells.

Since it had been observed that the effect of aminopterin in inhibiting mitosis was overcome when fibroblasts and osteoblasts grew from one to three days in tissue culture, the inactivation of the antimetabolite by a variety of tissues was investigated. It was found that no activity of aminopterin persisted after twenty-four hours incubation with fragments of frontal bone of chick embryos. Homogenates of this tissue failed to inactivate aminopterin, however. Similar incubation of mouse leukemia cells and of intestinal mucosa with aminopterin in Tyrode solution for twenty-four hours brought about loss of activity. Bone marrow from normal mice did not inactivate aminopterin. The author points out that bone marrow of the mouse is particularly susceptible to the effects of aminopterin and states the opinion that this may be the result of the inability of this tissue to inactivate the antagonist. Other tissues which possess the ability to inactivate aminopterin may be presumed to be less affected and to gain release from mitotic inhibition as the antimetabolite is degraded.

These experiments are of great interest because they provide cytologic evidence of the site of action of folacin antagonists and demonstrate this to be the result of interference with a metabolic role of citrovorum factor or a closely related compound in cell division. Although a characteristic defect in mitosis occurs with a block at the stage of metaphase, no specific disturbance in nucleic acid metabolism has been pointed out by these studies.

THE SYNTHESIS OF ENZYMES

Growth of a living organism is inevitably accompanied by the synthesis and deposition of new proteins and fats, and to a lesser degree of carbohydrates. It is taken for

granted that these synthetic processes are catalyzed by enzymes. Knowledge of the mechanism of synthesis of the enzymes is rather scant. Enzymes are themselves proteins, and the mechanism of protein synthesis is obscure. The synthesis of enzymes is of special interest because of the existing evidence that genes exert their influence by regulating the synthesis of enzymes.

M. M. Daly and A. E. Mirsky (*J. Gen. Physiol.* **36,** *243 (1952)*) have studied the turnover of enzymes in the pancreas with the primary aim of studying protein synthesis. The pancreas is particularly suited to such studies since the principal products of synthesis—the digestive enzymes—are relatively easily identified. Furthermore, the tissue may be studied either at rest or may be stimulated to active synthesis at will. They found that in nonsecreting mouse pancreas the dry, fat-free material contained about 5 per cent trypsin, 5 per cent chymotrypsin, 5 per cent amylase, 2 per cent carboxypeptidase and 0.2 per cent ribonuclease. The amount of lipase could not be estimated because this enzyme has not been purified. Digestive enzymes, therefore, make up about 20 per cent of the dry, fat-free material of the resting pancreas. Feeding or pilocarpine stimulation caused a marked drop in enzyme content of the tissue, amounting in some experiments to a loss of 90 per cent of the total enzyme weight. Restoration of the original high level of enzyme content required three to six hours.

Desoxyribonucleic acid (DNA), found largely in the cell nucleus, remained constant during secretion and synthesis of enzymes. This might be expected since the amount of DNA per nucleus is thought to be constant for any one tissue, and the pancreatic cells do not disintegrate during secretion. J. Brachet ("Embryologie Chimique," *Masson et cie, Paris (1947)*) and T. Caspersson ("Cell Growth and Cell Function," *W. W. Norton, New York (1950)*) have concluded, largely on the basis of microspectrophotometric studies of tissue slices, that there is a correlation between the concentration of ribonucleic acid (RNA) and the rate of protein synthesis. Daly and Mirsky found no changes in pancreatic RNA (determined chemically) during secretion and synthesis of the enzymes. They also offer calculations which indicate that the amount of total cellular protein does not change during the secretion and synthesis of enzymes. If this is true (and perhaps more refined experiments are needed to settle the point), the rapid extrusion of enzyme from the secreting cell is accompanied by the equally rapid synthesis of a nonenzyme protein which is then gradually transformed into the several pancreatic enzymes.

Yeast cells have long been a favorite system for the study of enzyme synthesis. One important reason for this preference lies in the fact that certain yeasts can form "adaptive enzymes." While a certain strain of yeast may normally ferment the sugar glucose, it may, when exposed to galactose, gradually synthesize the enzymes required to ferment this sugar which it does not normally encounter. It is important in such experiments to decide whether the synthesis of a new enzyme has actually occurred or whether the galactose substrate has caused the glucose-fermenting cells to be replaced by galactose-fermenting mutants. L. S. Baron, S. Spiegelman, and H. Quastler (*J. Gen. Physiol.* **36,** *631 (1953)*) and others have recently presented evidence that cells which normally ferment glucose can indeed form the galactose-fermenting enzyme. This has been accomplished by subjecting the yeast cells to x-rays at a dosage of 380,000 roentgens. Such intense irradiation was found to kill 99.996 per cent of the cells. The resulting nonviable cells were still capable of synthesizing the galactose-fermenting enzyme. Normal and irradiated cells were equally capable of utilizing the nitrogen of ammonium sulfate for the synthesis of amino acids and enzyme pro-

tein. It would seem that irradiated yeast preparations constitute an excellent system for the study of the mechanism of enzyme synthesis and of factors required for such synthesis.

J. L. Koppel, C. J. Porter, and B. F. Crocker (*J. Gen. Physiol.* **36,** *703 (1953)*) have developed a method for the study of enzyme synthesis which appears to be a splendid refinement of older technics. A strain of *Escherichia coli* was grown on a lactose-containing medium to induce the synthesis of beta-galactosidase, an enzyme capable of splitting the sugar galactose from certain organic combinations. Further enzyme synthesis was stopped by the addition of chloromycetin, which was shown not to interfere with the activity of enzyme already formed. The cells of *E. coli* were then treated with a phage to disrupt the cell membrane so that the cell contents could be assayed for the newly formed galactosidase without interference by the cell membrane. Toluene treatment of the cells, while not actually rupturing the membrane, also facilitated enzyme assay. The concentration of enzyme was determined from the extent of cleavage of a synthetic substrate, ortho-nitrophenol-beta-D-galactoside.

Sodium, potassium and chloride ions were found to be nonessential for enzyme synthesis. In fact, they could actually inhibit enzyme formation, sodium being more potent in this respect than potassium. The optimum pH for enzyme synthesis was near 7. As expected, the amount of available nitrogen influenced the amount of enzyme synthesized, the maximum rate of formation being attained when the medium in which the cells were growing contained ammonium sulfate in concentrations between 0.015 and 0.030 molar. Asparagine or glutamine served as well as ammonium sulfate as nitrogen sources. On the other hand, equivalent amounts of nitrogen supplied as peptone, tryptose, Casamino acids, or yeast extract considerably enhanced enzyme synthesis.

A variety of sugars share with lactose the ability to evoke the synthesis of galactosidase. All of them, however, contain the galactose moiety. Structural analogues such as glucose inhibit the formation of this enzyme, although glucose in very low concentration can stimulate enzyme synthesis presumably by serving as an energy source. Enzyme synthesis can also be enhanced by other energy sources such as succinic acid.

The induction of enzyme formation by structurally related compounds (as shown by Koppel *et al.*, as well as by other investigators) and the inhibition of such synthesis by structural analogues argues that the inductor must specifically combine with the enzyme-forming catalyst. Apparently enzyme synthesis is catalyzed by other enzymes.

In a second paper by the same group, C. J. Porter, R. Holmes, and B. F. Crocker (*J. Gen. Physiol.* **37,** *271 (1953)*) reported in further detail the proof that the time course of enzyme formation is linear providing nitrogen source, oxygen supply, etc., are not limited. They advance the theory that the cell synthesizes a series of polypeptide fragments which can be assembled into definitive cellular proteins (such as enzymes) according to the inherited potentialities of the cell and the environmental conditions existing at the moment. They postulate that for every enzyme which the cell is capable of forming there exists a genetically determined factor which together with a suitable inductor causes the selection of the proper polypeptides and their condensation to form the specific enzyme.

ERRATUM

Vol. 12, No. 5, page 157, column 2, paragraph 1, line 8: 85 g. should read 35 g.

NOTES

Letter to the Editor

Dear Sir:

Might I draw to your attention an error in the review "The Lipotropic Effect of Threonine" (*Nutrition Reviews* **12,** *151 (1954)*)? Where the effect of tryptophan supplementation is discussed in the last paragraph in the right hand column on page 151, the levels of tryptophan tested are stated as 0.5 and 1 per cent. These figures should read 0.05 and 0.1 per cent.

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Food Consumption and Expenditure in Britain

The British Ministry of Food instituted in 1940 a "National Food Survey," a permanent organization which served as a watchdog over British food consumption during the difficult war years. Emphasis was given to the "vulnerable" groups of the population, and the spectacular success of rationing and distribution schemes in Britain was due in no small measure to this inspired and efficient group. This organization, under the able leadership of Norman C. Wright, has since enlarged its scope and for a number of years, starting in 1950, has studied a national sample representative of the whole population. The results of these studies not only make possible a clearer view of the over-all nutritional picture in the United Kingdom, but also permit comparisons between the diets of households of different "social classes" (economic groups) and of different family composition.

The survey for 1951 ("Domestic Food Consumption and Expenditure 1951," *Annual Report of the National Food Survey Committee, Her Majesty's Stationery Office, London (1953)*) represents an improvement in statistical methods over the 1950 survey. A monthly survey was used rather than one conducted during two months of each quarter. Larder stocks were not weighed unless they consisted of home-grown foods; a longer, more continuous, and more representative sample was thus obtained.

The year 1951 was characterized in Britain by diminishing supplies and higher prices, with a resultant decline in meat consumption (20 per cent), eggs (12 per cent), and pulses and nuts (8 per cent). Consumption of sugar, oils and fats, and fish increased. As compared to prewar years, supplies of dairy products and potatoes were high, of meat were low; other staples were at similar levels.

In terms of nutrients the average diet compared very favorably with standards based on the 1950 Recommendations of the British Medical Association. Caloric intake stayed at 100 per cent throughout the year; intakes of protein, iron, calcium and riboflavin were slightly above 100 per cent. Dietary contents of vitamins A, B, and niacin were in the 120 to 150 per cent range, while that of ascorbic acid varied between a "low" of 162 per cent (April–May) and a high of 347 per cent (July–August). Calories were derived from protein in the proportion of 12.5 per cent, fat contributed slightly above 35 per cent, and carbohydrates about 52 per cent. Food price indexes rose by about 8 per cent.

Four social classes were defined according to the income of the head of the family. Classes A, B, and C all had between 3.3 and 3.7 persons per family on the average. The poorest class (D) was subdivided into the group of "old age pensioners" (1.4 persons) and the rest of the class (2.8 persons). As could be expected, as one descended in the economic order, less and less money was spent on food, although this expense represented a larger and larger proportion of the total income. The absolute expense was about one-third higher for

the richest group than for the poorest group. The proportion of income allocated to food was four times higher for class D than for class A.

Proportions of calories contributed by protein, fat and carbohydrate were similar for all groups, slightly more protein and fat being consumed by the wealthier classes. Milk, eggs, vegetables, sugar, and especially fruit consumption were definitely increased with family income. In terms of nutrients, dietary amounts of calories, protein, calcium, iron, vitamins A, B, riboflavin, niacin and ascorbic acid were all correlated with income, the correlation being particularly spectacular in the case of calcium, riboflavin and vitamin A.

When data were analyzed from the point of view of family size, it was found that food expenses per head decreased sharply when the size of the family group increased. This decrease was translated into smaller per caput supplies of all foodstuffs except milk and sugar in families with greater numbers of children. Intakes of protein, calcium, riboflavin and ascorbic acid were particularly affected.

A study of this nature represents a model of the type which the Food and Agriculture Organization has tried to promote for its member nations. It constitutes an essential tool for economic planning based on real social needs. As an illustration, such a survey demonstrates the degree of efficacy of the enlightened British policy on milk in minimizing economic differences as regards the availability of this important foodstuff.

Recent Nutrition Books

Food Selection and Preparation. Marion Deyoe Sweetman and Ingeborg MacKellar. 4th edition. John Wiley & Sons, Inc., 440 Fourth Avenue, New York 16, N. Y.; Chapman & Hall, Ltd., London, 1954. Pp. 645. Price: $6.50.

Zein: An Annotated Bibliography, 1891–1953. Dorothy M. Rathmann. Mellon Institute Bibliographic Series Bulletin No. 7. Published by the Mellon Institute, 4400 Fifth Ave., Pittsburgh 13, Penna., January 1954.

Methods of Biochemical Analysis, Volume I. Edited by David Glick. Interscience Publishers, New York. Pp. 509. Price: $9.50.

Vegetable Fats and Oils. ACS Monograph No. 123. E. W. Eckey. Reinhold Publishing Company, New York. Pp. 789. Price: $16.50.

The World's Food. M. K. Bennett. Harper & Brothers, New York. Price: $4.00.

Survey of Food and Nutrition Research in the United States of America, 1952–1953. Prepared by the Food and Nutrition Board, National Research Council. Published by the U. S. Department of Agriculture, Washington, 1954. Price: $1.75. Copies may be purchased from the U. S. Government Printing Office, Washington 25, D. C.